

Plasmid Backbone

FIGURE 2A

FIGURE 2B

GTTAACTACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATA TGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTC CGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGA $\tt TGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCC$ ${\tt TCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAG}$ GCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCA $\tt CTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAG$ ACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTG ATTTACCCCGGTTGATAATCAGAAAAGCCCCAAAAACAGGAAGATTGTATAAGCAAATATTTAAATTGTAAACGTTAATA AAATCAAAAGAATAGCCCGAGATAGGGTTGAGTGTTGTTCCAGTTTGGAACAAGAGTCCACTATTAAAGAACGTGGACTC GAAAGGAAGGAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGTCACGCTGCGCGTAACCACCACA ${\tt GAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTCACATGTAATGTG}$ ${\tt TCCACCGGTAGCGCCAACCGGCTCCGTTCTTTGGTGGCCCCTTCGCGCCACCTTCTACTCCTCCCCTAGTCAGGAAGTTC}$ ${\tt ACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCGCAGGGGGCGCCCGGTTCTTTTTGTC}$ ${\tt TGTCATCTCACCTTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCT}$ TGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCGCCAGGCTCAAGGCGCGCATGCCCGACGGCGATG ${\tt ATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAAAATGGCCGCTTTTCTGGATTCATCGACTGT}$ ${\tt GGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCTTCCCGATTCGCAGCGCATCGCCTTCTTATCGCCTTCTTGACGAGT}$ ${\tt TCTTCTGAGGGGATCGATCCGTCCTGTAAGTCTGCAGAAATTGATGATCTATTAAACAATAAAGATGTCCACTAAAATGG}$ $\tt GTGGGGGTGGGGTTAGATAAATGCCTGCTCTTTACTGAAGGCTCTTTACTATTGCTTATGATAATGTTTCATAG$ $\tt TTGGATATCATAATTAAACAAGCAAAACCAAATTAAGGGCCAGCTCATTCCTCCCACTCATGATCTATAGATCTATAGA$ ${\tt TCTCTCGTGGGATCATTGTTTTTCTCTTGATTCCCACTTTGTGGTTCTAAGTACTGTGGTTTCCAAATGTGTCAGTTTCA}$ TAGCCTGAAGAACGAGATCAGCAGCCTCTGTTCCACATACACTTCATTCTCAGTATTGTTTTGCCAAGTTCTAATTCCAT ${\tt CAGAAGCTGACTCTAGATCTGGATCCGGCCAGCTAGGCCGTCGACCTCGAGTGATCAGGTACCAAGGTCCTCGCTCTGTG}$

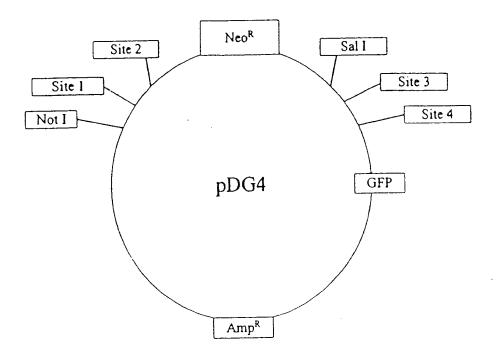


FIGURE 3A

FIGURE 3B

GTTTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGG CCCGCCTGGCTGACCGCCCAACGACCCCCCCCCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGA $\tt CTTTCCAATGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGT$ AGCTGGTTTAGTGAACCGTCAGATCCGCTAGCGCTACCGGTCGCCACCATGGTGAGCAAGGGCGAGGAGCTGTTCACCGG AGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCGAGGTGAAGTTCGAGGGCGACAACCACTACCTGAGGACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTC $\tt GTGACCGCCGGGGATCACTCTCGGCATGGACGAGCTGTACAAGTCCGGACTCAGATCCACCGGATCTAGATAACTGAT$ AAATGAATGCAATTGTTGTTAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTC TCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGGTATTATCCCGTGTTGACGCCGGGCAAGAGCAACTCGGTC GCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTA TACCAAACGACGAGCGTGACACCACGA1GCCTGTAGCAATGGCAACGATGCGCAAACTATTAACTGGCGAACTACTT $\tt CGCGTTAAATTTTTGTTAAATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAATCAAAAGAAT$ $\tt GTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACCTCAAGAACTC$ GGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTG GGAAACAGCTATGACCATGATTACGCCAAGCTACGTAATACGACTCACTAGGCGGCCGCGTTTAAACAATGTGCTCCTCT $\tt GCGCTTTAGCAGCCCCGCTGGCACTTGGCGCTACACAAGTGGCCTCTGGCCTCGCACACATTCCACATCCACCGGTAGCG$ ${\tt GCCATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCACAACA}$ $\tt CTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTCCTGTCATCTCACCT$ ${\tt TGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCG}$ ACCACCAAGCGAAACATCGCATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGACGAA GAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCGCCAGGCTCAAGGCGCGCATGCCCGACGGCGATGATCTCGTCGTGAC $\tt CTCGTGCTTTACGGTATCGCCGCTCCCGATTCGCAGCGCATCGCCTTCTTATCGCCTTCTTGACGAGTTCTTCTGAGGGGA$ ${\tt TCGATCCGTCCTGTAAGTCTGCAGAAATTGATGATCTATTAAACAATAAAGATGTCCACTAAAATTGGAAGTTTTTCCTGT}$ ${\tt GGGATTAGATAAATGCCTGCTCTTTACTGAAGGCTCTTTACTATTGCTTTATGATAATGTTTCATAGTTGGATATCATAA}$ TTTAAACAAGCAAAACCAAATTAAGGGCCAGCTCATTCCTCCCACTCATGATCTATAGATCTATAGATCTCTCGTGGGAT GAGATCAGCAGCCTCTGTTCCACATACACTTCATTCTCAGTATTGTTTTGCCAAGTTCTAATTCCATCAGAAGCTGACTC ${\tt ACGACACGGACACGCAAATTAATTAAGGCCGGCCCGTACCCTCTAGTCAAGGCCTTAAGTGAGTCGTATTACGGACTGG}$ $\tt CCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCC$ AGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCTTCGC TTGGTAATAAAGCCCGCTTCGGCGGGCTTTTTTTT

FIGURE 3B (Continuted)

			, , , , , , , , , , , , , , , , , , ,
			Sequence after digestion
Annealing		Sednence	
site			3.
	5' tgtgctccto	screttggettgettcaa3'	3, 19190100100100000000000000000000000000
4	3 , ಇಂಇಂಡ್ರಪ್ಪನ್ನೆ	gaaaccgaacgaagg	E. crantfolfatotagottagoccaa3'
(5, ctggttctt	ctggttcttgtctggcttggccaa 3	3. (1989) (1987) (1987) (1987) (1987) (1987)
7	3' gaccaagaa	gaccaagaacagaccgaaccgggcc	רא מעמט די דאַ דאַ דאַ דּיַרָ דְּיַרְ דְּיִרְ בָּיִרְ בַּיִּרְ בַּיִּרְ בַּיִּרְ בַּיִּרְ בַּיִּרְ בַּיִּרְ בַּי
	5, agtectes	gatcotogototgtgtccgttgaa 3'	S' ggtactagatagagagagagagagagagagagagagagag
3		gagggagggagggaactt5	5
	5 66555		5, tttgcgtgtcctgtgtcgtcgaa 3'
	S, tttgcg	tttgcgtgtcctgtgtcgtcgaa	3. tt 5.
4	3' aaacgc	acgcacaggacacagcagcacc	
	,		

FIGURE 4

				Sequence after digestion	_
Annealing		Sequence			
site			-	3.	-
1	50.0	AAtgtgctcctctttggcttgcttCCGC 3	n m	Tracacgaggagaaaccgaacgaagg 5'	-
	า	רמכמכם	u	3,	•
·	20.	AActggttcttgtctggcttggCCCGC	n m	Ttgaccaagaacagaccgaaccggg 5'	-
7	- M	Trgaccaayaacayaacayaa		. 8	
	5 -	AAggtcctcgctctgtgtccgttGAGCT 3		Trecaqqaqegagacacaggeaac	
ท	'n	Trecaggagegagaeacaggeaac		8	
	5	AAttgcgtgtcctgtgtcgtcGAGCT	 n m	Traaacgcacaggacacagc	- 10
,	<u>-</u>	Traaacgcacaggacacagcag			

FIGURE 5

FIGURE 6

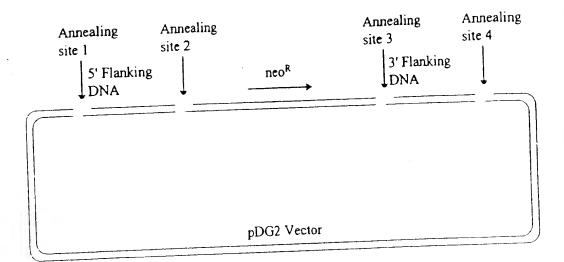
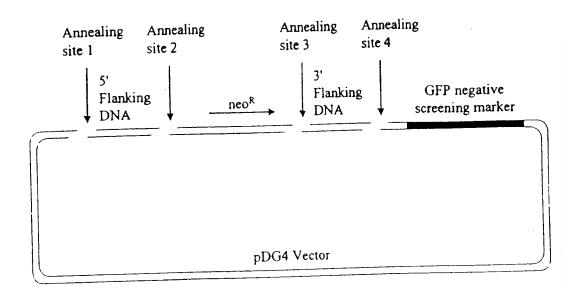


FIGURE 7



TTCCTGACAAGACTATGTCCACTCAGGAGCCCCAGAAGAGTCTTCTGGGTTCTCTCAACTCCAATGCCAC TTCCTCAGCCTAGGGCTGGTGAGTCTGGTGGAGAATGTGCTGGTTGTGATAGCCATCACCAAAAACCGCA ACCTGCACTCGCCCATGTATTACTTCATCTGCTGCCTGGCCCTGTCTGACCTGATGGTAAGTGTCAGCAT CGTGCTGGAGACTACTATCATCCTGCTGCTGGAGGTGGGCATCCTGGTGGCCAGAGTGGCTTTGGTGCAG CAGCTGGACAACCTCATTGACGTGCTCATCTGTGGCTCCATGGTGTCCAGTCTCTGCTTCCTGGGCATCA TTGCTATAGACCGCTACATCTCCATCTTCTATGCGCTGCGTTATCACAGCATCGTGACGCTGCCCAGAGC ACGACGGGCTGTCGTGGGCATCTGGATGGTCAGCATCGTCTCCAGCACCCTCTTTATCACCTACTACAAG CACACAGCCGTTCTGCTCTGCCTCGTCACTTTCTTTCTAGCCATGCTGGCACTCATGGCGATTCTGTATG CCCACATGTTCACGAGAGCGTGCCAGCACGTCCAGGGCATTGCCCAGCTCCACAAAAGGCGGCGGTCCAT CCGCCAAGGCTTCTGCCTCAAGGGTGCTGCCACCCTTACTATCCTTCTGGGGATTTTCTTCCTGTGCTGG GGCCCCTTCTTCCTGCATCTCTTGCTCATCGTCCTCTGCCCTCAGCACCCCACCTGCAGCTGCATCTTCA AGAACTTCAACCTCTTCCTCCTCCTCATCGTCCTCAGCTCCACTGTTGACCCCCTCATCTATGCTTTCCG CAGCCAGGAGCTCCGCATGACACTCAAGGAGGTGCTGCTGTGCTCCTGGTGATCAGAGGGCGCTGGGCAG AGGGTGACAGTGATATCCAGTGGCCTGCATCTGTGAGACCACAGGTACTCATCCCTTCCTGATCTCCATT TGTCTAAGGGTCGACAGGATGAGCTTTAAAATAGAAACCCAGAGTGCCTGGGGCCAGGAGAAAGGGTAAC GCAAGGGTCAGACCACAGGCTCCTGAAGAGCTTCACCTCTCCCCACCTACAGGCAACTCCTGCTCAAGCC (SEQ ID NO: 19)

Targeting Vector (5' arm; 200 bp flanking neo insert):

CCGACAACAACATGAAGTGAATCAGAAGCTGGGGGCTGATACCACCTGGAGCTGCAG CCTCCACAGACCGCTTCCTACTTCCTGACAAGACTATGTCCACTCAGGAGCCCCAGAA GAGTCTTCTGGGTTCTCTCAACTCCAATGCCACCTCTCACCTTGGACTGGCCACCAACC AGTCAGAGCCTTGGTGTCTGTATGTG (SEQ ID NO: 20)

Targeting Vector (3' arm; 200 bp flanking neo insert):

GACTACTATCATCCTGCTGCTGGAGGTGGGCATCCTGGTGGCCAGAGTGGCTTTGGTG CAGCAGCTGGACAACCTCATTGACGTGCTCATCTGTGGCTCCATGGTGTCCAGTCTCT GCTTCCTGGGCATCATTGCTATAGACCGCTACATCTCCATCTTCTATGCGCTGCGTTAT CACAGCATCGTGACGCTGCCCAGAG (SEQ ID NO: 21)